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Low Risk of Venous Thrombosis in Two Families With Combined Type I Plasminogen Deficiency and Factor V R506Q Mutation

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Two families with type I plasminogen deficiency and APC resistance are reported. The proposita of family A suffered from ischemic stroke when taking estrogen-progesterone-containing oral contraceptive. Several hemostatic challenges in the past (ovariectomy, appendectomy, and two pregnancies) were without thrombosis. Plasminogen activity and antigen (60 and 58%, normal range 72–136 and 69–135%, respectively) were reduced, and an increased APC resistance (APC-SR = 1.55; normal range 1.8–3.00) associated with G → A change at 1,691 nucleotide position in exon 10 of FV gene (FV Leiden) was observed. The asymptomatic son had isolated plasminogen deficiency (activity 57% and antigen 60%) whereas the asymptomatic daughter had isolated APC resistance (APC-SR = 1.61) and FV Leiden mutation. The proposita of family B, referred for superficial thrombophlebitis, had low plasminogen levels (activity 55% and antigen 53%) and APC resistance (APC-SR = 1.5) whereas the asymptomatic mother and the brother had isolated APC resistance (APC-SR = 1.62 and 1.8, respectively) and the asymptomatic father isolated plasminogen deficiency (activity 61% and antigen 62%). These data suggest that the combination of plasminogen deficiency and APC resistance probably does not significantly increase the risk of venous thrombosis. However, larger experience with additional cases is needed to definitely assess the magnitude of thrombotic risk in these families. Am. J. Hematol. 57:344–347, 1998. © 1998 Wiley-Liss, Inc.

Key words: inherited thrombophilia; APC resistance; FV Leiden; plasminogen deficiency; thrombosis

INTRODUCTION

Poor anticoagulant response to activated protein C (APC) is by far the most frequent inherited disorder associated with thrombophilia [1]. A point mutation in exon 10 of factor V gene, causing Arg506 to be replaced by Gln at the APC cleavage site (FV Leiden), has been detected in more than 90% of patients with APC resistance [2,3]. Several studies have demonstrated that FV Leiden is present in 15–25% of patients with antithrombin III, protein C, or protein S deficiency [4–6], all well-established risk factors for venous thrombosis. Thus, the concept of thrombophilia as multigenic disorder, with the association of multiple hemostatic defects greatly increasing the risk of thrombosis, is now emerging.

No other defects of clotting or fibrinolytic system have been universally established as risk factors for inherited thrombophilia. However, since FV Leiden has a high prevalence in the population, the association of FV Leiden with these controversial defects should not be rare and could substantially increase the risk of thrombosis. We report here two families with type I deficiency of plasminogen associated with APC resistance due to FV Leiden. The combination of the two abnormalities does not seem to greatly increase the risk of thrombosis.

MATERIALS AND METHODS

Family A

The propositus was referred at age 49 for laboratory evaluation after ischemic stroke. Prior to the cerebrovascular accident, she had been taking an oral contraceptive

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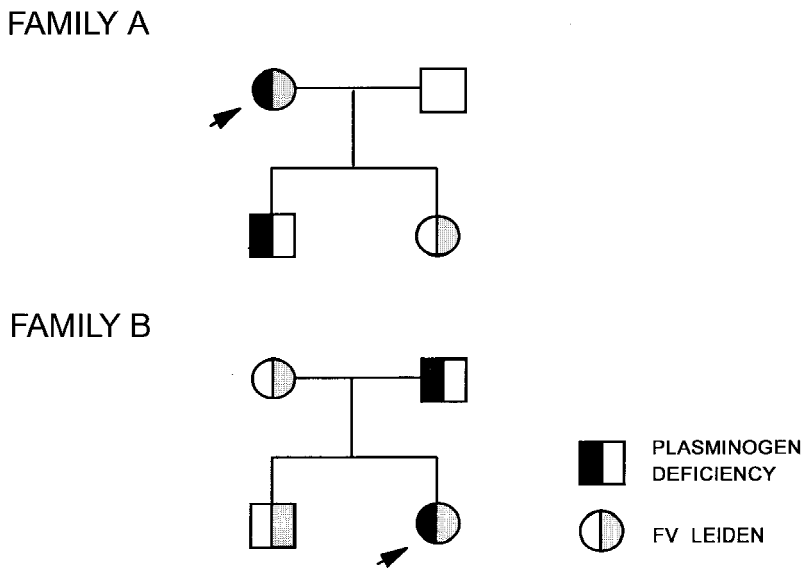

Fig. 1. Pedigree of the two families. The arrow indicates the propositus of each family.

for 1.5 years for bleeding associated with uterine myoma. In the past, she underwent right ovariectomy at age 35 and appendectomy at age 39 without mishap. The two pregnancies occurred without thrombotic complications. Both the parents died of neoplastic disease, but they were not reported to have suffered from thromboembolic events. The propositus's son was born in 1970. He had liver biopsy at age 20 for evaluation of chronic hepatitis C. He did not undergo surgical interventions. The propositus's daughter was born in 1971. At the moment of first evaluation, she had been taking an estroprogestinic pill for 2 years before for menstrual abnormalities. She had neither surgical interventions nor pregnancies. The family tree is shown in Figure 1.

Family B

The propositus was referred at age 28 for evaluation of an episode of superficial thrombophlebitis of left leg after erysipelas. At age 20, she underwent appendectomy without thrombotic complications. She did not have pregnancies. Her 67-year-old father had an appendectomy at age 33 and hemorrhoidectomy at age 39 without mishap. At age 61 he had a car accident with four rib fractures, without thromboembolic complications. The 64-year-old mother had two pregnancies and a cholecystectomy at age 37 without history of thrombosis. The 34-year-old propositus's brother had three neurosurgical interventions for damage of ulnar and radial nerves of both arms after motor vehicle and work accident. He had no history of thrombosis. The maternal grandmother, deceased, was reported to have suffered from superficial thrombophlebitides. The family tree is shown in Figure 1.

METHODS

Coagulation Studies

Blood for coagulation studies was collected in polystyrene tubes and anticoagulated with sodium citrate (1:10). Plasma was obtained by centrifugation at 2,500*g* for 15 min, snap-frozen in liquid nitrogen, and stored at −30°C within 2 h after collection. Antithrombin III, protein C, and plasminogen were assayed as previously described [7]. Free protein S antigen was assayed by an ELISA using a monoclonal antibody (Asserachrom free PS, Stago, Asnieres, France). Resistance to APC was measured as previously described [8], using an ACL 300 coagulometer (Instrumentation Laboratory, Milan, Italy). Results were expressed as APC-sensitivity ratio (APC-SR) defined as the ratio of the aPTT with APC to the aPTT without APC. Inter-assay coefficiency of variation was 4.6% for a normal plasma in a series of 70 measurements. Plasminogen antigen was measured by rocket immunoelectrophoresis using 1% goat antiserum (Behring, Scoppito, Italy) Anticardiolipin antibodies (ACA) were assayed by an ELISA technique (Boehringer Mannheim, Milan, Italy).

FV Leiden Mutation

Leukocyte DNA was pelleted after cell lysis with Triton X-100 and stored at −80°C. Analysis of FV Leiden mutation was carried out using PCR amplification of exon 10 of FV and digestion with MnlI restriction endonuclease [2]. The pattern of digestion was visualized under UV after electrophoresis on ethidium-bromide agarose gel.

TABLE I. Hemostatic Indices and FV Leiden in the Families

Subjects	AT-III (%)	Protein C (%)	Protein S (%)	Plasminogen[a] Act/Ag (%)	APC[a] (SR)	FV Leiden[b]
Family A						
Proposita	102	94	88	60/58	1.55	QR
Son	94	95	101	57/60	2.29	RR
Daughter	90	87	95	87/91	1.61	QR
Family B						
Proposita	89	79	101	55/53	1.51	QR
Mother	100	97	97	88/99	1.62	QR
Father	106	94	97	61/62	2.24	RR
Brother	90	92	95	98/97	1.8	QR
Normal range (N = 40)	78–134	71–136	70–145	72–136/69–135	1.8–3.0	RR

[a]The results represent the average of two determinations obtained on two separate occasions.
[b]Presence (QR) or absence (RR) of Arg506Gln substitution.

RESULTS AND DISCUSSION

Table I shows the results of laboratory investigation of the two families. The propositus of family A showed an increased APC resistance and low levels of plasminogen activity and antigen, compatible with type I plasminogen deficiency. The son had isolated plasminogen deficiency whereas the daughter had isolated APC resistance. The presence of FV Leiden mutation was associated with the phenotype APC resistance (not shown). Also the propositus of family B showed increased APC resistance and type I plasminogen deficiency. Her mother and the brother showed isolated APC resistance, whereas the father showed isolated plasminogen deficiency. FV Leiden mutation cosegregated with the phenotype APC resistance also in this family (not shown). In all the families, APTT, fibrinogen level, ACA titer, antithrombin III, proteins C and S were in the normal range (data not shown). Interestingly, the propositus of family A had several possible triggering conditions in the past without venous thrombotic complications, whereas ischemic stroke occurred when taking an oral contraceptive. In women with APC resistance, the risk of venous thromboembolism associated with oral contraceptives greatly increases [9]. Recently, it has been demonstrated that FV Leiden increases the risk of myocardial infarction in young women [10] and that it may also be associated with the early presentation of arterial thrombotic events [11]. Thus, FV Leiden could have contributed to arterial thrombosis in the propositus of family A. The presence of other defects possibly responsible for stroke, including ACA, was ruled out. The daughter, carrying isolated APC resistance, was on an oral contraceptive, which was discontinued as soon as the diagnosis of APC resistance was made. The propositus of family B had superficial venous thrombosis associated with erysipelas. Surgical challenge was without thrombotic complications. Only the maternal grandmother had suffered from superficial thrombophlebitis. Thus, probably this minor tendency to thrombosis was associated with APC resistance trait and FV Leiden, present in the mother, who, however, did not suffer from any thrombotic complications despite several hemostatic challenges (pregnancy and surgery).

The role of partial plasminogen deficiency as a risk factor for thrombosis is still a matter of debate. Sartori et al. [12] reported that 1/4 individuals with plasminogen deficiency suffer from thrombosis whereas Shigekiyo et al. [13] failed to demonstrate a significant correlation between type I plasminogen deficiency and thrombosis in 21 affected individuals from two Japanese families. In the present families, the individuals with isolated plasminogen deficiency did not suffer from thrombosis, despite the fact that in one several triggering conditions had been present. FV Leiden mutation carries a significant risk of thrombosis. This risk has been estimated to be 80-fold, and 7-fold higher in patients with homozygous and heterozygous mutation, respectively, in comparison to normal individuals [14]. However, even homozygotes may remain free from thrombosis despite the presence of triggering factors [15]. Obviously, the presence of additional, yet uncovered, risk factors may contribute to the heterogeneity of symptoms. Very recently, Zuger et al. [16] reported a single family with associated plasminogen deficiency and FV Leiden. Even in that family, patients with isolated plasminogen deficiency were asymptomatic as in the present ones, whereas two subjects with combined deficiency developed recurrent thromboembolism. However, the presence of pulmonary embolism in a relative with isolated APC resistance led these authors to conclude that FV Leiden mutation was likely to be the major hereditary risk factor in that family [16]. Our two propositi had arterial thrombosis developed during oral contraceptive treatment and superficial thrombophlebitis associated with infection. Both had hemostatic challenges in the past without thrombosis. Even though these

data do not allow a definite conclusion, it appears that the combination of these abnormalities probably carries a much lower risk of thrombosis in comparison to that observed in patients with combined FV Leiden and antithrombin III, protein C or S deficiency [17]. In thrombophilic families with protein C deficiency and FV Leiden, for example, a history of thrombosis was present in 31% of individuals with isolated protein C deficiency, in 13% of those with only FV Leiden, and in 73% of those with both defects [18].

Thus, the present data suggest that combined deficiency of plasminogen and heterozygous FV Leiden may not represent a strong risk factor for venous thromboembolism. However, more families should be reported to firmly establish the clinical significance of this association.

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